Microfluidic sorting system based on optical waveguide integration and diode laser bar trapping

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Effective methods for manipulating, isolating and sorting cells and particles are essential for the development of microfluidic-based life science research and diagnostic platforms. We demonstrate an integrated optical platform for cell and particle sorting in microfluidic structures. Fluorescent-dyed particles are excited using an integrated optical waveguide network within micro-channels. A diode-bar optical trapping scheme guides the particles across the waveguide/micro-channel structures and selectively sorts particles based upon their fluorescent signature. This integrated detection and separation approach streamlines microfluidic cell sorting and minimizes the optical and feedback complexity commonly associated with extant platforms.

Introduction

Microfluidic systems for life science research and diagnostics will be significantly enhanced by the introduction of practical, miniature cell particle sorting techniques. Conventional fluorescent activated cell sorting (FACS) and flow cytometry techniques require cumbersome instrumentation, and are not readily available on the micro-scale. 1 While the precision and throughput of these tools are high, experimental demands are often far surpassed by their capabilities. As biochemical analyses continue to increase in sophistication and single-cell microbiology techniques become more common, 2,3 traditional FACS instruments will be complemented by devices tailored for small samples. High-throughput is a feature of conventional FACS instruments, which is non-essential for emerging lab-on-a-chip and micro total analysis systems (μTAS) 4 applications. Indeed, microfluidic platforms have begun to proliferate as small-sample alternatives for high-throughput screening needs. 5,6 The strength of these platforms rely in their ability to seamlessly integrate multiple step, serial processes in parallelized arrays and small sample sizes. Cell sorting components of μTAS, for both research and clinical applications, must therefore dovetail smoothly with complementary on-chip processes. To enable compact or even portable instrumentation, it is also desirable that cell sorters do not dominate the function of the microsystem nor encumber its performance with excessive hardware or operating demands.

Numerous microfluidic cell sorting schemes based upon electrophoresis, 7,8 dielectrophoresis 9 and flow switching 10,11 have been devised. These approaches generally act upon externally processed fluorescent signals received from an external source coupled to the chip. Detection and sorting are therefore necessarily independent of one another, which presents timing, alignment and calibration difficulties.

Optical trapping 12–15 has been introduced as an alternative actuation scheme for microfluidic FACS. The non-invasive and sterile nature of optical tweezers is particularly appealing for use with biological particles. Employing optical radiation for both identifying target cells and sorting them suggests the possibility of complete process integration. This potential has gone largely undeveloped, however, due to the use of separate optical trains with distinct wavelengths for the detection and the trapping. This design is dictated mostly by the use of single beam optical traps, which require high numerical aperture (NA) lenses as well as elaborate and often quite costly beam steering techniques. Reliance upon single beam traps has eliminated not only the effectiveness of process integration, but also the possibility of parallel sorting. We have recently demonstrated the successful manipulation of particles using diode laser bars as an alternative to single beam traps. Optical gradients formed by diode laser bars are line-shaped and are effective at simultaneously manipulating multiple particles in conjunction with microfluidic flows. Additionally, diode laser bars require minimal optics for focusing and do not need to be translated during sorting. To further reduce the fabrication and process control complexity associated with microfluidic cell sorters, we have integrated diode laser bar optical trapping with monolithic waveguides. This represents a significant step towards complete process integration and has produced a novel method for sorting fluorescent cells and particles on the basis of their optical signature within microfluidic systems.

Our ability to completely integrate sorting and detection relies upon direct waveguide writing using ultra-fast laser pulses, which produce index of refraction changes within materials. 16 Tightly focusing a femtosecond pulsed laser into a block of fused silica produces a material change that locally increases the index of refraction of the glass. 17–20 By translating the focus through the glass, a line of higher refractive index surrounded by a lower index material—a waveguide—is created. The waveguide transports single or...
multi-mode beams of light with minimal loss. Utilizing waveguides in coordination with microfluidic channels, we induce fluorescence in particles above the individual output of the guide. Because the excitation light is localized at the output of the waveguide, only colloids or cells occupying a position directly above the output spot will fluoresce. Placing a band pass or rejection band filter in front of a CCD camera enables the excitation wavelength to be blocked while detecting the emission from a particle located at the waveguide output. In this way, distinct flashes of a specific wavelength are produced as individual fluorescent particles pass over the waveguide outputs. This technique gives us a parameter with which to sort particles that is compatible with many standard, well-developed fluorescent-labeling protocols. A particle can be sorted into a desired output stream by positioning it with the diode laser bar and identifying its fluorescence characteristics at the release point for the output, with the waveguide.

**Experimental**

To manipulate individual particles we focus and align a 100 μm by 1 mm diode laser bar, centered at a wavelength of 980 nm, within a microfluidic channel. The laser output is relayed one-to-one into the sample to create an identical image of the diode bar and thus a 100 μm by 1 μm trap. The microchannel and contents are imaged with a CCD camera connected to a monitor and camcorder to capture the images. A schematic of the complete optical train can be seen in Fig. 1. For these experiments the diode laser bar output power is approximately 500 mW; polystyrene microspheres ranging in size from 4 μm to 10 μm are employed. Particles dyed with two distinct fluorescent dyes are used: Crimson Red (625 nm absorption peak; 645 nm emission peak) and Nile Red (535 nm absorption peak; 575 nm emission peak). Two excitation lasers of appropriate wavelength, a 0.95 mW HeNe (λ = 632.8 nm) and a 5 mW green laser pointer (λ = 532 nm), are utilized. A photodiode or CCD camera with an optical filter captures the fluorescence signal, and a shutter blocks the trapping beam for particle release. The signal sensor and shutter are controlled using computer feedback routines run on a PC with LabView.

For sorting experiments with conventional optics, non-waveguided excitation light is focused upon the sample with a simple 20 × , 0.4 NA, microscope objective. For sorting experiments with integrated waveguiding, excitation lasers are coupled directly into optical waveguides. The waveguides used are 6 μm in diameter. A single input beam is split into 4 output beams of approximately equal intensity that are spaced 30 μm apart. The circular mode waveguides are machined into a 50.8 mm by 25.4 mm by 12.7 mm block of fused silica (refractive index of 1.457 at λ = 633 nm) using femtosecond pulses. The resulting index of refraction change is approximately 6 × 10⁻³, with an estimated emission NA of 0.15 (Sample #AOT460 Translume, Inc.).

Microfluidic channel networks were created in poly(dimethylsiloxane) (PDMS), using well established soft lithography techniques. The fluid is forced through the channels by creating a vacuum at the outlets with syringes. The dimensions and orientation of both the trapping beam and hydrodynamic focusing network used to align the sample particles into a streamline that intersects the edge of the trapping region can be seen in Fig. 2. In this long-exposure micrograph, one can clearly see striated streamlines traced by fluorescent particles. The sample particles, cells or colloids, are delivered from an upstream channel and focused into a continuous line of particles; the dark streamline in Fig. 2. The particles then intersect the line trap and are pushed, by the flow, across the channel.

Sorting rate calibrations were performed experimentally and trapping force estimates are presented. Experimental calibrations are conducted with a static line trap configuration in conjunction with a microchannel that flows at four fixed angles (θ) relative to the laser. The particle velocity (Vp) at which the trapping force (Ftrap) is exceeded by Stokes’ drag upon the particle is measured experimentally using a microchannel configuration, as shown in Fig. 2. The force exerted by the line trap is one-dimensional and has no lateral...

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**Fig. 1** Schematic of integrated microfluidic sorting system based on optical waveguide integration using diode laser bar trapping. The trapping laser is aligned over the waveguide outputs.
optical gradient. As a result, the particle’s translation along the trap is unconstrained. For these experiments, the laser was operated at 450 mW and 4.2 μm polystyrene colloids were sent through a 25 μm deep channel.

**Results and discussion**

The central cell sorting method uses a diode laser bar trap to translate sample particles across streamlines in a microfluidic device. Due to the laminar nature of microfluidic flows, particles precisely follow the streamlines that they are in. Exploiting this makes it possible to easily predict the fate of a particle by positioning it into a given streamline.

Focusing the diode laser bar within the microfluidic channel and orienting the resulting trap line at an angle with respect to the direction of flow forms the cell sorter. The end of the trap line is aligned so that it protrudes into the sample streamline. This configuration serves to modify a trapped particle’s axial motion by introducing a force perpendicular to the streamline. The particles encountering the trap are therefore translated along the trap line as if they were in an “optical conveyor belt”; the particles enter at one end of the trap, flow down the line, and are released as they reach the downstream terminus. To release the particles before they reach the end of the trap, all or part of the beam may be temporarily blocked, sending the particles into any given streamline along the length of the trap. To introduce particle sorting on the basis of fluorescence, an external laser is also focused within the microfluidic channel. By aligning the trap line with the path of the excitation laser, particles will express their fluorescent signal at a single location as they pass through the focus of the excitation beam while being translated along the trap line. By combining this knowledge of the fluorescent properties of the particles with the precise streamline that the particle is in, a level of control is attained that can be exploited for sorting.

A simple feedback mechanism, which converts the measured emission flash into a command to shutter the beam, releases the particle from the trap.

Fig. 3 demonstrates this single parametric sorting technique. Here, a mixture of labeled (Nile Red fluorescent dye) and unlabeled 4 μm colloids are sorted based strictly on their fluorescence signal. A 532 nm excitation beam is focused to a single spot within the channel. A fluorescent particle enters the trap (Fig. 3a) and fluoresces (Fig. 3b). The particle’s position is monitored by optical microscopy as it continues its path along the trap until it has reached the designated separation streamline (Fig. 3c). The beam is then shuttered, releasing the particle into the desired output stream (Fig. 3d–3f). As is shown, a single fluorescent colloid is picked out from a group of non-fluorescent colloids and sent to a specific output channel. This technique functions for sorting at a single point with a single wavelength, but an alternate technique is needed that can be scaled to a larger and more diverse number of particles. Accommodating and controlling selective multi-parametric distributions of particles throughout the microfluidic networks demands the ability to both identify multiple fluorescent labels and automatically track particle position. We accomplish both by integrating optical waveguiding networks into the sorting scheme.

Our waveguides have the unique ability to accept a single input beam and split it to an arbitrary number of outputs with little loss. This multifunctional beam splitting and waveguiding capability may be used to track the position of particles while simultaneously multiplexing the excitation radiation for sorting into multiple, parallel channels. Because the waveguides are only 6 μm in diameter and may be spaced as close as desired (although crosstalk is a concern when the waveguides get within evanescent range), we additionally envision the potential for multi-parametric sorting with several individual waveguides, each transporting a distinct wavelength. Combining multiplexed waveguides with the diode laser bar’s ability to trap over a large linear distance, and thus a large number of streamlines, overcomes the scaling limitations of conventional beam steering optics. To demonstrate the
integration of waveguiding and trapping, we begin by aligning the diode bar trap over four waveguide outputs. This ensures that fluorescence emitted by trapped particles will be localized over individual waveguides. Fig. 4 illustrates this technique. Aligning the outputs of the waveguide below narrow streamlines associated with individual microfluidic outlet channels, and immediately inhibiting the trapping laser when fluorescence is detected, allows for precise particle placement. A helium–neon laser was coupled into our single input, 4-output waveguide array, and used to excite fluorescence in 10 μm diameter Crimson Red labeled colloids. In this simple demonstration, shuttering the beam releases fluorescent colloids into outlet 1 while the non-fluorescent colloids are directed into channel 2. In Fig. 4b the first fluorescently labeled particle brightly fluoresces over a waveguide output. The emission radiation is detected using a photodiode and the trapping beam is subsequently blocked, causing this colloid to be sent into outlet 1. The second particle does not display fluorescence and thus is allowed to traverse the entirety of the trap, whereupon it is released into its respective streamline and exits into output 2. Thus, the sorting of particles displaying distinct optical characteristics is enabled by diode bar optical trapping in conjunction with integrated waveguide-coupled excitation light.

This integrated approach to microfluidic FACS streamlines sorting feedback control, and therefore increases the throughput potential of optically actuated platforms. Higher sorting rates can be pursued through a number of routes, including beam splitting for parallel processing, multiwavelength analysis and independent control over portions of the beam. To first explore the fundamental limitations of the approach, however, we have tested the trap strength by monitoring the “failure velocity” of a flowing particle from the trap. The point at which the trapping beam would no longer trap and hold the particles was considered to be the failure velocity. An experimental value of the optical trapping force was found by plotting the failure velocity against the angle. Due to the relatively small size of the particles with respect to the channel height, we assumed that Stokes drag ($F_{\text{Drag}} = 6\pi \eta rv$) described the force of the fluid on the particle, despite the parabolic velocity profile of the fluid. We then balanced the optical and fluid forces and rearranged them to predict trap failure velocity as a function of angle, yielding $V = F_{\text{Trap}}/(6\eta r \cos(\theta))$, where $r$ is the particle radius (2 μm) and $\eta$ the viscosity (0.855 cP). Using water as a medium, we fit the experimental data to this simple model to obtain an estimate for the one-dimensional force exerted by the diode bar line trap, finding a trapping force of approximately 6 pN. The force of the laser is assumed to be constant, allowing the model to be fit to the data using least squares. Fig. 5 shows the experimental data and the model prediction for failure velocity and demonstrates that as the angle increases the maximum fluid velocity increases significantly. Each value comes from the same geometry in different channels. However, the added speed available from a higher angle is balanced against a loss of range. The distance by which a particle can be translated is

![Fig. 4](#) Images of a non-fluorescing colloid and a fluorescing colloid being sorted into different output streamlines, labelled 1 and 2 in frame a. Channel edges and particle positions are marked for clarity. Frames a-c show the path of the fluorescing colloid going into the outlet labelled 1. In frame b the colloid is fluorescing over the waveguide output aligned with outlet 1. Frames d–f show the non-fluorescing colloid path. Note the flash in frame b.

![Fig. 5](#) Graph of experimental results showing trap failure velocity as a function of angle of the 100 μm diode laser bar trap with respect to the flow direction.
related to the angle of the trap by \( D = L \cos(\theta) \), where \( D \) is the lateral distance a particle can be translated and \( L \) is length of the trap. Thus, to sort efficiently in fast flow environments, fewer waveguide outputs may be placed within the trapping zone. A compromise must therefore be made between the number of sorting parameters and the speed at which a particle can be trapped. Analyzing each cell individually in the current sorting scheme requires the entirety of the single trapping beam to be blocked and thus, cells must be at least 100 \( \mu \text{m} \) apart. Sorting at a 65-degree angle would result in 5 cells per second, or 1,000,000 cells in about 2 days. To overcome this limitation to high throughput multiparametric sorting, we intend to multiplex the trapping and excitation beams. Only a fraction of the available diode bar laser power (the optical trapping laser) was used, indicating that a single diode laser bar capable of 3 W could be split to manipulate objects over a vast array of channels. Utilizing waveguides rather than focused light sources allows us to localize and multiplex the vast array of excitation wavelengths by simultaneously coupling independent waveguide inputs. Manipulative tasks within the microfluidic system are a straightforward process using this integrated laser diode and waveguide geometry.

**Conclusion**

An integrated optically actuated FACS platform has been demonstrated. The use of femtosecond pulsed lasers as a critical element in the robust optical platform described here by eliminating the need for fiber optics and significantly decreasing alignment demands. Additionally, the waveguides provide precise spatial control over the localization of fluorescence excitation, which enables single particle detection and positional tracking. Successful integration of fluorescent excitation and optical trapping eases the demands of process control and portends the development of this sorting technique into a viable microfluidic FACS platform.